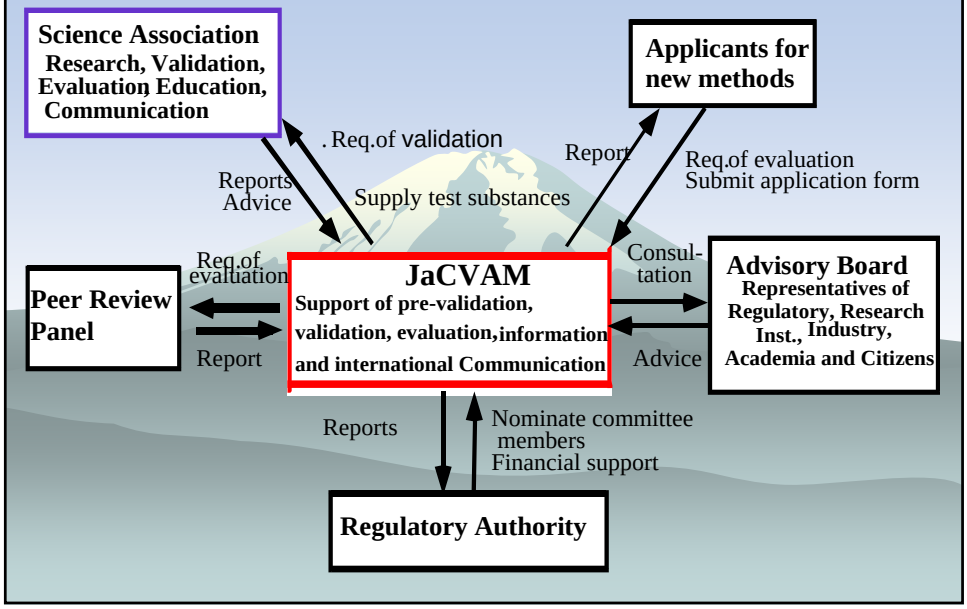
Organization and Mission
JaCVAM

November, 2006

Framework of evaluation on alternative methods (Ohno draft)



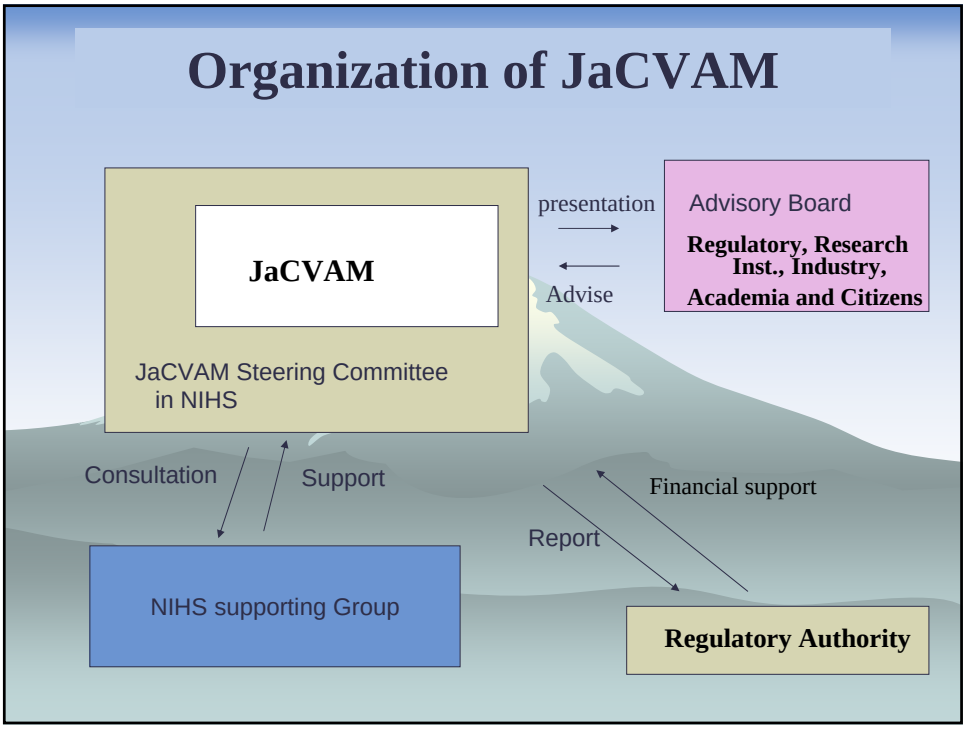
Establishment of JaCVAM

Date	**November, 2005**
Affiliation	**National Institute of Health Sciences (NIHS) , National Center for Biological Safety and Research (NCBSR), Div. of Pharmacology**
Founder	**Dr. Yasuo Ohno (NIHS)**
Director	**Dr. Hajime Kojima**

Mission of JaCVAM

- Development of new test method
- Validation: Proposal and support of validation (secretary and logistics in the validation management team)
- Peer review: Proposal and support of peer review (manage the peer review committee as a leader and the peer review panel as a secretary)
- International cooperation
- Planning and reporting (annual, validation, peer review)

Organization of JaCVAM



Members of Steering Committee

- **Chair Toru Inoue (NIHS,NCBSR)**
  Yasuo Ohno (NIHS)
  Kenichi Nakazawa (NIHS)
  Hajime Kojima (JaCVAM)
  Hiroshi Itagaki (JSAAE)

Observer Mitsuteru Masuda (JaCVAM)

Mission of Steering Committee

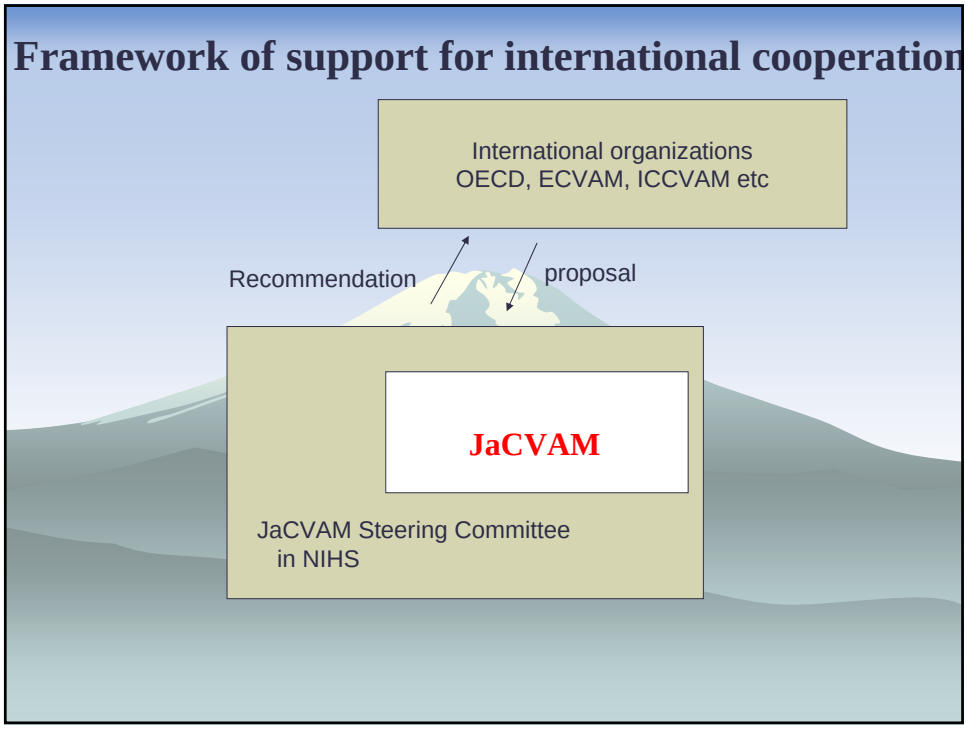
- **Determination of need for validation or peer review of new or revised test method**
- **Determination of international cooperation**
- **Selection of members of advisory board**
- **Selection of members of peer review panel**
- **Consult with support members in NIHS**
- **Check plans and reports of JaCVAM**

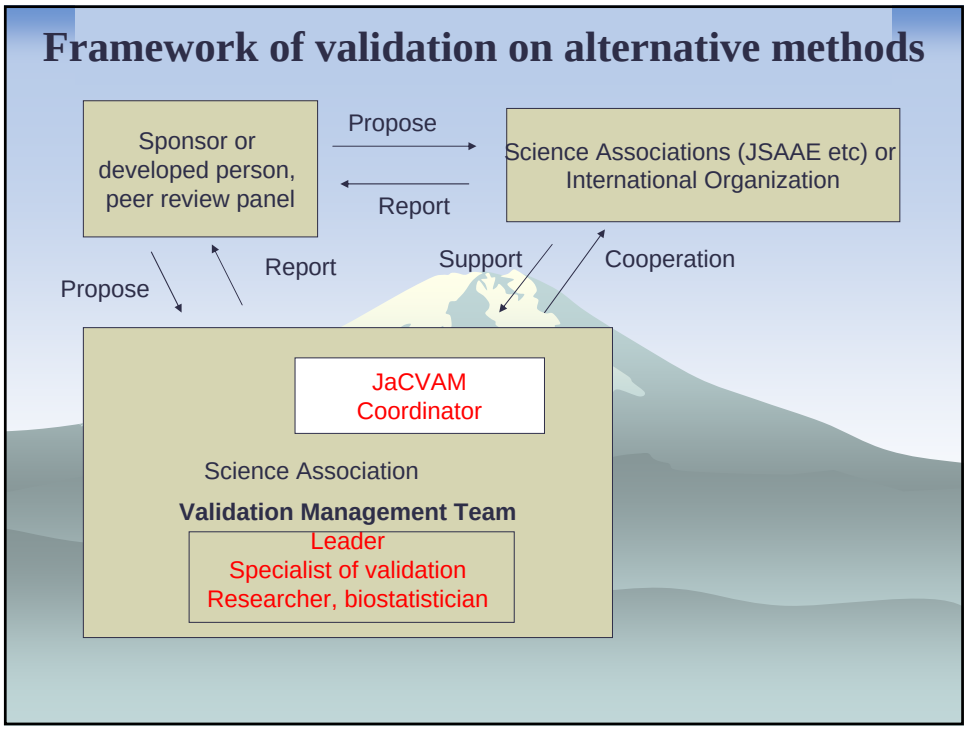
Members of Advisory Board

- Hiroshi Ono (JST)
- Hiroyoshi Ninomiya (JALAS)
- Yasuyuki Sakai (JSAAE)
- Kiyoshi Nishioka (Dermatologist)
- Fumio Sagami (JPMA)
- TBD (JCIA)
- Keiko Yamasaki (Animal Welfare Network)
- Toshiro Nakagaki (MHLW)
- Observer: Yasuo Ohno (NIHS), Hajime Kojima (JaCVAM)

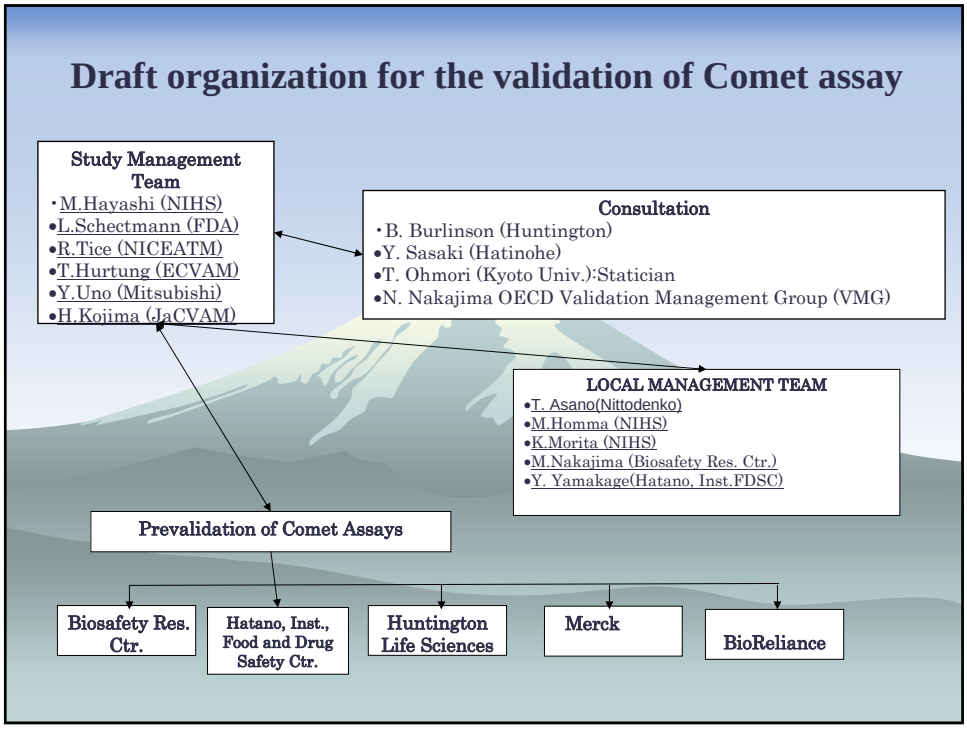
Mission of Advisory Board

- Advice on the operation of JaCVAM
- Check of the annual work of JaCVAM
- Advice on the funding for JaCVAM
- Support communication of JaCVAM with industries, science associations, regulatory agencies and public


Framework of support for international cooperation
International organizations
OECD, ECVAM, ICCVAM etc
Recommendation
proposal
JaCVAM
JaCVAM Steering Committee
in NIHS


Framework of validation on alternative methods
Sponsor or
developed person,
peer review panel
Propose
Report
Science Associations (JSAAE etc) or
International Organization
Propose
Report
Support
Cooperation
JaCVAM
Coordinator
Science Association
Validation Management Team
Leader
Specialist of validation
Researcher, biostatistician

Draft organization for the validation of Comet assay

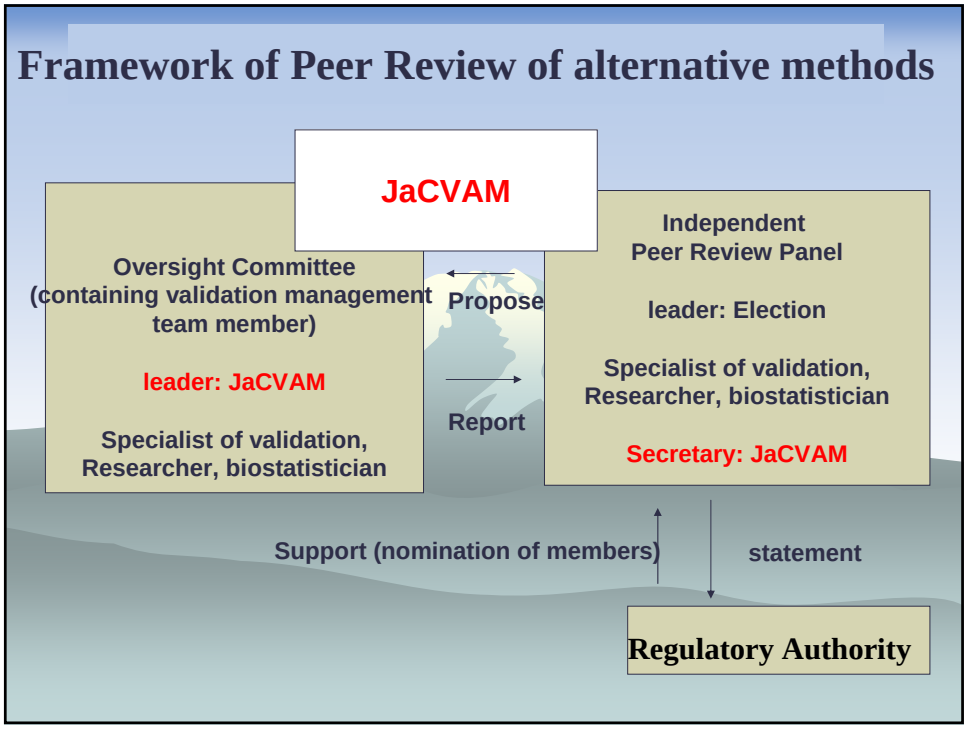


Mission of Validation Management Team

For reliability and repeatability

- **Planning**
- **Selection of participating Laboratories**
- **Management of Test Substances (selection, blinding, distribution)**
- **Data management (data sheet preparation and data collection, cleaning and analysis)**
- **Reporting**

Framework of Peer Review of alternative methods



Mission of Oversight Committee

For evaluation of test method

- Collect of references
- Preparing of draft report
- Proposal of validation
- Advise for further research

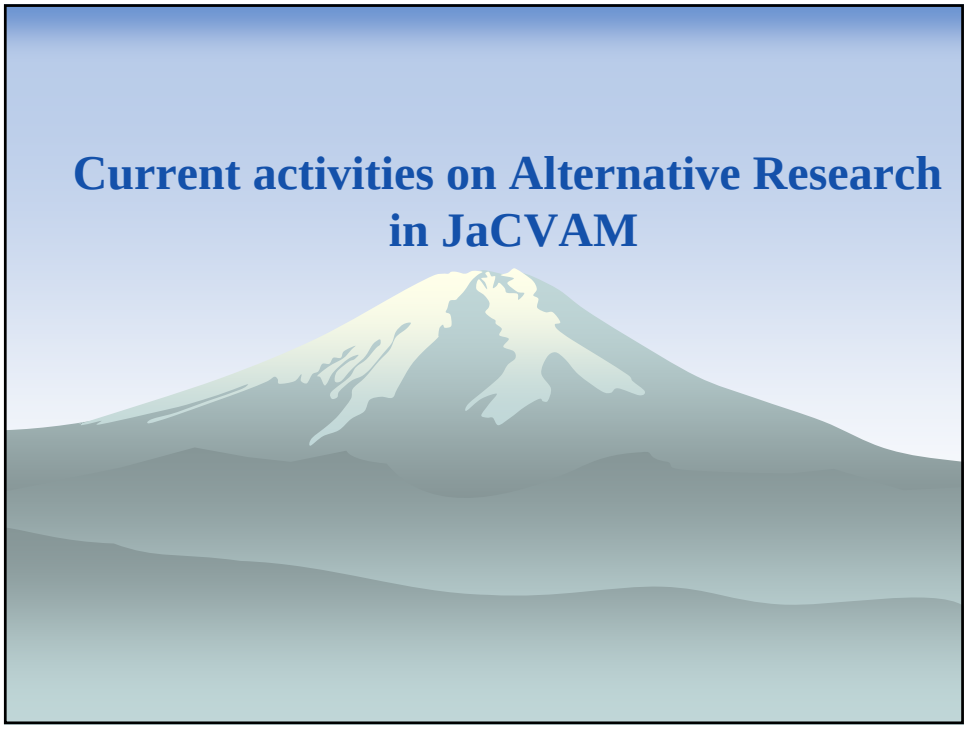
Mission of Peer Review Panel

- Peer review of new or revised test method based on the report of oversight committee
- Propose further validation, if necessary
- Prepare report and statement on the test method for regulatory agencies

Peer Review Panel

Toru Inoue (NIHS)
Makoto Hayashi (NIHS)
Noriho Tanaka (JSAAE)
Takemi Yoshida (JST)
Isao Yoshimura (Biostatistician)
Masako Mizoguchi (Dermatologist)
Fumio Sagami (JPMA)
TBD(JCIA)
Hiroshi Onodera (PMDA)
Hajime Kojima (JaCVAM)

Current activities on Alternative Research in JaCVAM



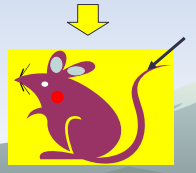
Current validation and peer review in Japan

Test method	Material	Current activities
Photoxicity	Yeast-RBC	Peer Review in progress
Skin sensitization	LLNA-DA	Validation in progress
	LLNA-BrdU	Validation in progress
	h-CLAT	Pre-validation in progress
Corrosivity	Culture model	Peer Review in progress
Skin irritation	Culture model	Planning on Peer Review
Endocrine disrupter	Lumi-cell, CER-estrogen reporter assay	Planning on Validation
Mutagenicity	Comet assay (in vivo or in vitro)	Planning on Validation

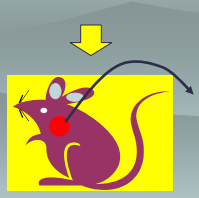
LLNA (Local lymph node assay)

OECD guideline 429 (2002)

Day1、2、3
Chemical application



Day6
Apply ^{3}H-TdR in vain of Tail



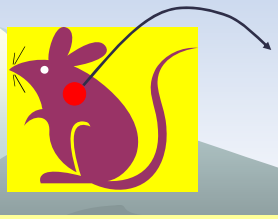
5Hr later
Extract Lymph node, measure of
^{3}H-TdR uptake
(cell growth)

GPMT、Buehler

- Deduction
- Refinement
- Low cost& short term

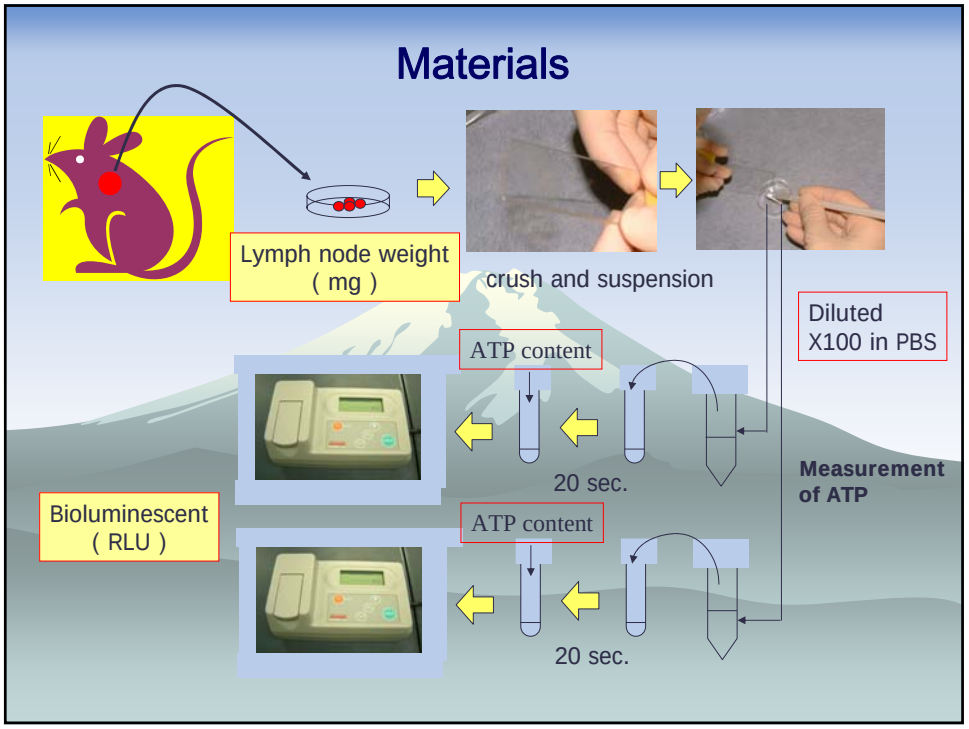
LLNA-DA

At 1、2、3、7 days、apply chemicals
1 hr Before treatment, apply 1% SLS **solution**



mouse CBA/JN ♀ 8～12w

At 8 day, extract lymph node,
Measure lymph node weight and ATP levels/mice (n=4)



Validation Plan of LLNA-DA

Date:April-July/2006

Participated lab.:10, 2-3 tests/Lab.

Chemical used: Total 12, 4-6substances/Lab.

- 3 chemicals were examined by all 10 experimental laboratories while 9 chemicals were each tested by 3 different laboratories. Chemicals with the fixed 3 doses were distributed to each laboratory coded to disguise their type. The value of 3 was set as the cut-off point of the stimulation indices (SI), which summarize the ATP amount.

Director: Dr. T. Ohmori (Kyoto Univ.)

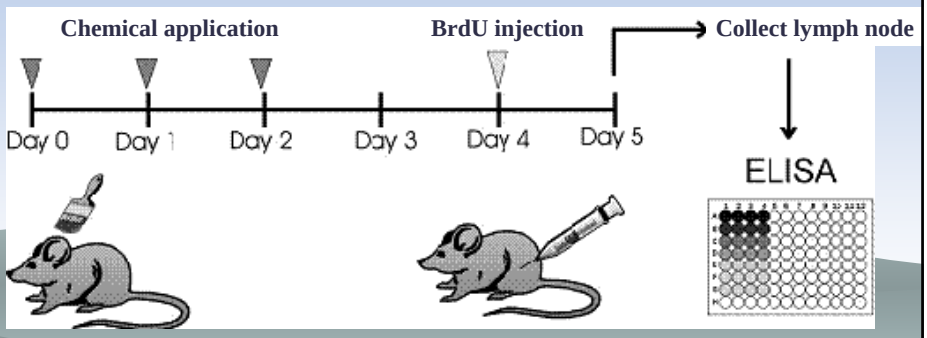
Organizer: Validation Committee in JSAAE

Support: JaCVAM (Selection, coding & supply of chemical and materials)

Results and Discussion

The results for the 3 chemicals examined by all laboratories and 5 of the other 9 chemicals were consistent and have small variances in the SI. There were 4 chemicals which produced inconsistent results between 3 laboratories. 2 chemicals showed the clearly dose response relationships. On the other hand, for the other 2 chemicals it seemed that the type of solvent in these chemicals caused the large variations. Sensitivity, specificity and concordance of the LLNA-DA compared to the GPMT/BT were 87.5% (7/8), 100% (3/3) and 90.9% (10/11), respectively. We conclude that, considering the published data of the LLNA, the results from this study are acceptable as a catch-up validation study, at least within the range of examined chemicals.

LLNA - BrdU



Validation Plan of LLNA-BrdU

Date:October-December/2006

Participated lab.9, 2-3 tests/Lab.

Chemical used: Total 12, 4-6substances/Lab.

Director: Dr. Hajime Kojima (JaCVAM)

Organizer: Validation Committee in JSAAE

Support: JaCVAM (Selection, coding & supply of chemical and materials)

Comparison with results obtained from alternative methods in GPMT

Class in GPMT	Chemicals	DEREK	TOPKAT	Peptide-binding assay	h-CLAT	LLNA-BrdU	LLNA-DA	LLNA
Positive (5 chamicals)	Cinnamic aldehyde	Positive	Positive	Positive	Positive	Positive	Positive	Positive
	2,4-Dinitrochlorobenzene	Positive	Positive	Positive	Positive	Positive	Positive	Positive
	α -Hexylcinnamic aldehyde	Positive	Positive	Positive	Positive	Positive	Positive	Positive
	Formaldehyde	Positive	Positive	Positive	Positive	Positive	Positive	Positive
	p-Phenylenediamine	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Negative(3 chamicals)	Lactic acid	Negative	Negative	Negative	Negative		Negative	Negative
	Resorcinol	Positive	Positive	Negative	Positive		Positive	Negative
	Sodium lauryl sulfate	Negative	Positive	Negative	Negative		Negative	Positive

Thank you for your kind attention!